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(54) Title: **METHOD OF DETECTING NEUROLOGICAL DISEASE OR DYSFUNCTION**

(57) Abstract

This invention relates to a method of detecting and diagnosing neurological disease or dysfunction using antibodies against a neurological form of Pancreatic Thread Protein (nPTP). Specifically, this invention is directed to a method of diagnosing Alzheimer's Disease, Down's Syndrome, and other neurological diseases or dysfunctions by using monoclonal antibodies, combinations of those monoclonal antibodies or nucleic acid probes to detect nPTP. The invention also relates to a recombinant DNA molecule encoding PTP and to the substantially pure form of nPTP. The invention additionally relates to a method of diagnosing pancreatic disease using antibodies against Pancreatic Thread Protein.

BOVINE PTP: cDNA AND AMINO ACID SEQUENCES

GGCAGGAGAGCTGCTCCACACCTCACAGACACBAATGCTGCCCTCCCTGGGCGCTCCCGAG
M L P S L G L P R
ACTGTCTGGATGCTGCTCTCCTGCTGATGCTCCTGTCTCAGATCCAAGGGGAAAATTC
L S W M L L S C L M L L S Q I Q G E N S
CCAAAGGAAGTGCCTCTGCAAGGATCAGCTGTCCCTCAGGTTCCATGGCCTATAGGTC
Q K E L P S A R I S C P S G S M A Y R S
TCACTGCTATGCCCTGTGTTAAACACCCAAACCTGGATGGATGCAGATATTGCCCTGCCA
H C Y A L F K T P K T W M D A D I A C Q
GAAGAGGCCCTCGGGACATCTGTGTCTGTGCTCAGTGGGGCTGAGGAATCCITCGTGGC
K R P S G H L V S V L S G A E E S F V A
CTCCTTGSTTAGGAACAACCTGAACACCCCAATCAGACATCTGGATTGGGCTCCATGACCC
S L V R N N L N T O S D I W I G L H D P
CACAGAGGGCTCTGAGGCCAATGCTGGTGGATGGGAATGGAATTAGCAATGACGCTCAA
T E G S E A N A G G W E W I S N D V L N
TTACGTTGCCCTGGGAGACAGATCCTGCTGCCATCTCAAGCCCTGGCTACTGTGGGAGTCT
Y V A W E T D P A A I S S P G Y C T G S L
CTCAAGAAGCTCAGGATATCTCAAGTGGAGAGATCATACTGCAATTTGAACCTTACCTA
S R S S G Y L K W R D H N C N L N L P Y
CGTCTGCAAGTTCACAGACTAGATCAGATGAGAAGTCAGCAGCCTGACTGGTGTGCAACT
V C K F T D
GGTGTGCAACTCATCATGGAAGTGGAACTAGGGATTCAGACCCCACTTGAAGGGGATAT
TCTTCTCACAGCCCAACCCCACTTCACTTGAACCTTCCCTCTCTCCAGACTCAAT
TCAGTCTCTTCTGTGTTCATTAACCTGACTTTGCAAGTTCACATAAATAATATTAGT
TTCTCTGCC

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TITLE OF THE INVENTION

METHOD OF DETECTING NEUROLOGICAL DISEASE OR DYSFUNCTION

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FIELD OF THE INVENTION

This invention relates to proteins associated with Alzheimer's Disease, Down's Syndrome, neural tube defects and pancreatic disease. The invention further relates to the genes encoding such proteins, immunodiagnostic and molecular diagnostic methods to diagnose these disease.

ABBREVIATIONS

For brevity, the following abbreviations are used throughout this application: Pancreatic Thread Protein (PTP); Neural Pancreatic Thread Protein (nPTP); Immunoradiometric Assay (IRMA); Monoclonal Antibody (mAb); Alzheimer's Disease (AD); Down's Syndrome (DS); Neurofibrillary Tangles (NFTs); and Paired Helical Filaments (PHFs).

20

BACKGROUND OF THE INVENTIONNEUROLOGICAL DISEASES

Alzheimer's Disease (AD) is the most frequent cause of dementia in the United States, affecting over two million individuals each year. It is a degenerative brain disorder characterized clinically by loss of memory, confusion, and gradual physical deterioration. It is the fourth most common

25

cause of death. The etiology of the disease is virtually unknown but has been attributed to various viruses, toxins, heavy metals, as well as genetic defects. The disease is at present incurable.

5 Until quite recently, AD was thought to account for relatively few of the cases generally classified as senile dementia. Other factors can lead to such a condition, including repetitious mild strokes, thyroid disorders, 10 alcoholism, and deficiencies of certain vitamins, many of which are potentially treatable. It can be appreciated, then, that a diagnostic test specific for AD would be very useful for the clinical diagnosis and proper clinical treatment of subjects presenting with symptoms common to all of these conditions.

15 The brains of individuals with AD exhibit characteristic pathological accumulations of congophilic fibrous material which occurs as neurofibrillary tangles (NFTs) within neuronal cell bodies, and neuritic (or senile) plaques. NFTs may also be found in the walls of certain cerebral blood vessels. The 20 major organized structural components of NFTs are paired helical filaments (PHFs). Qualitatively indistinguishable amyloid deposits also occur in normal aged brains but in much smaller numbers with restricted topographical distribution.

25 There has been considerable recent investigative activity regarding the characterization of proteins found in neuritic plaques and NFTs of AD and other neurologic diseases. One of the amyloid proteins initially described by Glenner et al. has been cloned and sequenced (Glenner et al., Biochem. Biophys. Res. Commun. 120:1131-1135 (1984); U.S. Patent No. 4,666,829). 30 The A4 amyloid protein found in neuritic plaques and blood vessels has been determined to be a component of a 695 amino acid precursor; a protein postulated to function as a glycosylated cell surface receptor (Masters et al., Proc. Natl. Acad. Sci. USA 82:4245-4249 (1985), Kang et al., Nature

325:733-736 (1987)). The gene coding for A4 is located on chromosome 21 (Kang et al., ibid.; G6ldgaber et al., Science 235:877-880 (1987); Tanzi et al., Science 235:880-885 (1987); St. George-Hyslop et al., Science 235:885-889 (1987)) but
5 apparently is not linked to the familial form of the disease (Van Broekhoven et al., Nature 329:153-155 (1987)). There appears to be little, if any, protein sequence homology between amyloid A4 and β protein, their higher molecular weight precursor, and nPTP described by the present invention
10 (see discussion below) (Gross et al., J. Clin. Invest. 76:2115-2126 (1985)).

A number of other proteins thought to be associated with the disease have been described, including Ubiquitin, ALZ-50, microtubular-associated proteins τ and MAP2, and neurofilament
15 protein (see, for example, Manetto et al., Proc. Natl. Acad. Sci. USA 85:4502-4505 (1988); Wolozin et al., Science 232:648-651 (1986); Selkoe, Neurobiol. Aging 7:425-432 (1986); Perry et al., in: Alterations of the Neuronal Cytoskeleton in Alzheimer's Disease, Plenum, New York, pp 137-149 (1987)).
20 More recently, a serine protease inhibitor called α_1 -anti-chymotrypsin has been found in AD amyloid deposits (Abraham et al., Cell 52:487-501 (1988)).

Until this time, there has been no useful diagnostic test for AD. A definitive diagnosis is possible only postmortem,
25 or during life through a brain biopsy, to reveal the presence of the characteristic plaques, tangles, PHFs, and other cerebrovascular deposits which characterize the disorder. Such an invasive surgical procedure is inherently dangerous and is therefore rarely utilized. As a result, the clinical
30 misdiagnosis of AD is estimated to be approximately 20%-30%.

Down Syndrome (DS) results in mental retardation and is associated with a variable constellation of abnormalities caused by trisomy of at least a critical portion of chromosome 21 in some or all cells. No single physical sign is diagnos-

tic and most stigmata are found in some normal persons. In rare patients, no chromosome abnormalities can be detected by routine cytogenetic analysis. Although DS can generally be detected pre- and post-natally by cytogenetic testing, an
5 alternative diagnostic test which measured a parameter other than a gross karyotypic alteration would be useful in identifying and verifying the presence of DS in a subject, either pre- or post-natally.

Neural tube defects refer to defects which develop in the
10 vertebrate embryo in a tube formed from differentiated middorsal ectoderm. In a developing fetus, the neural tube ultimately gives rise to the brain and spinal cord. Thus, defects in the neural tube often result in severe defects in these organs. For example, such defects could include
15 anencephaly, the absence of the cerebral and cerebellar hemispheres of the brain, spina bifida (absence of vertebral arches of the spinal cord through which the spinal membranes (with or without spinal cord tissue) may protrude), meningocele (protrusion of the brain or spinal cord membranes
20 through a defect in the skull or vertebral column), meningo-myelocoele (protrusion of the membranes and spinal cord through a defect in the vertebral column), or holoprosencephaly (failure of the forebrain to divide into hemispheres).

A simple prenatal diagnostic test, using amniotic fluid,
25 for example, which could detect neural tube defects would be very useful in determining prenatal or early postnatal treatment such as, for example, immediate postnatal surgical intervention.

30 PANCREATIC AND OTHER DISEASES

Acute pancreatitis or acute pancreatic injury may be caused by multiple factors including alcohol, penetrating peptic ulcer, gallstones, drugs, trauma, uremia, etc. Diffuse abdominal pain, nausea and vomiting, fever, tachycardia,

epigastric tenderness and rigidity are cardinal symptoms and physical findings. Often hemoconcentration and intravascular volume depletion are present. Total serum amylase activity of 3-5 times greater than normal has been the diagnostic anchor for such diseases, despite the lack of specificity of this test. Measurement of serum lipase has also been somewhat helpful in this regard. However, serum amylase and lipase may be elevated in this same range in a variety of serious and life-threatening illnesses, some of which are medical emergencies.

For example, it is well-known that serum lipase and total amylase activities may be elevated in perforated ulcer, intestinal obstruction, intestinal infarction, and renal insufficiency. In these conditions, where no pancreatic injury has occurred, the signs and symptoms may be quite similar to those of acute pancreatitis. The treatment of these extrapancreatic causes of elevated amylase and lipase activities, however, is quite different from that for pancreatitis. For example, surgery for acute pancreatitis is discouraged, whereas failure to perform surgery for intestinal infarction can have lethal consequences. Thus, the search for a more specific diagnostic test of acute and chronic pancreatic injury has great clinical significance.

It is therefore clear that a simple, standardized, and relatively inexpensive assay for diagnosing neural tube defects or pancreatic disease, as well as for specifically detecting DS and AD, would be an immensely useful diagnostic tool for the clinician and researcher alike.

30 PANCREATIC PROTEINS

Pancreatic Thread Protein (PTP) is found in great abundance in the acinar cells of the pancreas and reaches concentrations of 1-2 mg/ml in normal pancreatic fluid as measured by a monoclonal antibody (mAb)-based immunoradio-

metric assay (M-IRMA) (Gross et al., J. Clin. Invest. 76:2115-2126 (1985)).

5 PTP in its monomeric form has an apparent molecular weight of approximately 14 kilodaltons (kD), consists of a single polypeptide chain and is rich in aromatic amino acids. The protein has unusual solubility characteristics: it undergoes a pH-dependent fibril formation at pH's between 5.4 and 9.2. The protein forms long "thread like" structures of 7-10 nm (by electron microscopy) when pancreatic fluid is
10 allowed to stand for several hours at 4°C (Gross et al., J. Clin. Invest. 76:2115-2126 (1985)). Thus, PTP represents one of the major secretory products of the exocrine pancreas in man.

Another pancreatic protein called pancreatic stone
15 protein (PSP) has been described by one research group (DeCaro et al., Biochem. Biophys. Res. Commun. 87:1176-1182 (1979)). Based on amino acid sequence, PSP appears identical to PTP (DeCaro et al., J. Biochem. 168:201-207 (1987)). A similar protein has been identified in bovine pancreas (Gross et al.,
20 Proc. Natl. Acad. Sci. USA 82:5627-5631 (1985)).

One group of investigators recently found that treatment of highly pure PHFs with pronase removed a 9.5 kD and 12 kD fragment which included the τ microtubular protein (Wischik et al., Proc. Natl. Acad. Sci. USA 85:4506-4510 (1988); Wischik
25 et al., Proc. Natl. Acad. Sci. USA 85:4884-4888 (1988)). The insoluble core protein remaining following pronase digestion had repeating subunits to which a mAb was made. The mAb bound specifically to the core protein but did not bind the τ protein (Wischik et al., Proc. Natl. Acad. Sci. USA 85:4506-4510 (1988)). The solubility characteristics and physical
30 appearance (under electron microscopy) of PTP (Gross et al., J. Clin. Invest. 76:2115-2126 (1985)) and the PHF core protein (Wischik et al., Proc. Natl. Acad. Sci. USA 85:4506-4510

(1988); Wischik et al., Proc. Natl. Acad. Sci. USA 85:4884-4888 (1988)) are similar.

SUMMARY OF THE INVENTION

5 A need exists for a definitive diagnostic test which can be performed on individuals suspected of having, or being at risk for, AD, DS, and other neurological disease. The present invention satisfies such needs and provides further advantages.

10 The manner in which these and other objects are realized by the present invention will be apparent from the summary and detailed description set forth below.

15 Unless defined otherwise, various terms used herein have the same meaning as is well understood in the art to which the invention belongs. All cited publications are incorporated herein by reference.

20 Because of the insolubility of PTP at physiologic pH and the physical appearance of the fibrils by electron microscopy, the inventors saw a resemblance of PTP to some of the fibrils observed in neuritic plaques, NFTs, and particularly the PHFs, of AD, leading to their studies which resulted in the present invention.

25 The inventors have identified, by M-IRMA, high concentrations of a neural form of PTP, referred to as Neural PTP (nPTP) in AD and DS brain. nPTP has been found in all AD brains studied where characteristic neuropathologic changes of the disease exist. The saline- extractable soluble immunoreactivity shares at least three epitopes with the native pancreatic form of PTP and has a molecular weight of approximately 17 to 20 kD.

30 Quantitative measurements of nPTP immunoreactivity in various regions of AD brains revealed levels varying from 12 to 295 ng/gm tissue (Mean = 116 ng/gm tissue) compared to 1-11

ng/gm tissue (Mean = 5 ng/gm tissue) in comparable areas of control brains.

Immunocytochemistry performed with mAbs directed against the pancreatic form of PTP demonstrated that nPTP is localized within cells, within fine processes within the neuropil, or is extracellular in both AD and DS brains. Two types of cell contain nPTP: neurons and astrocytes. The affected neurons are the large pyramidal type which typically contain the NFTs well known in AD brain.

That nPTP accumulation within neurons is intrinsically important or integrally related to the evolution of AD lesions is corroborated by the presence of identical patterns of immunolabeling for nPTP in DS brains, but not in control brains. It is important to note that the same structural abnormalities of AD occur in brains of all middle-age individuals with Down's syndrome, whether or not they are demented. There is also a higher incidence of AD in family members of DS patients. Moreover, the regional differences in the densities of nPTP-containing neurons parallels the density distributions of NFTs in both AD and DS. This provides further evidence that nPTP is germane to the pathophysiology of AD. Whether nPTP accumulates within neuronal perikarya, as a result of aberrant cellular metabolism or transport is not yet known. Accordingly, one object of the present invention is to provide a relatively simple, sensitive, accurate, and painless diagnostic method for detecting AD, DS, or other neurological defects which involve incontinence of the bony coverings of central nervous system tissue, such as neural tube defects which would permit the escape of cerebrospinal fluid (CSF).

Another object of the present invention is to provide a highly specific assay for diagnosing and distinguishing AD and DS from other disorders. The assays described by the present invention are non-invasive, thus avoiding the painful and

often hazardous removal of brain tissue samples. In view of the immense numbers of individuals potentially afflicted with AD, for example, the assays taught by the present invention will be relatively inexpensive to administer.

5 An additional object of the present invention is to provide a method for early diagnosis of neural tube defects. Prenatal diagnosis of these defects would allow for corrective actions to be taken prenatally or early postnatally.

10 Another object of the present invention is to provide a diagnostic method for detecting acute or chronic pancreatic disease, using a combination of antibodies as taught herein.

Furthermore, the assays of the present invention are capable of being reduced to a standardized format, easily and quickly performed.

15 The present invention additionally pertains to assays for detecting the presence of nPTP in the biological fluids of a human subject using histology, imaging, immunoassays, and the like as diagnostic methods for determining the presence of AD, DS, and neural tube defects, as well as detecting the presence of PTP as a diagnostic method for determining the presence of pancreatic disease.

20 In particular, the invention includes a method for detecting and quantitating nPTP in a human subject, comprising:

- 25 (a) contacting a biological sample from a human subject that is suspected of containing detectable levels of nPTP with a molecule capable of binding to the nPTP; and
 (b) detecting the molecule bound to the nPTP.

30 The invention additionally includes the method as above, wherein the molecule is selected from the group consisting of:

- (a) an antibody substantially free of natural impurities;
 (b) a monoclonal antibody; and
 (c) a fragment of (a) or (b);

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- (d) a polynucleotide probe derived from the recombinant bovine PTP of this invention; and
- (e) a polynucleotide probe derived from recombinant human PTP of this invention.

5 The invention additionally includes the method as above, wherein the detecting molecule is detectably labeled and where a combination of such molecules is used.

10 The invention additionally includes a method for determining the presence of a condition in a human subject, said condition including, but not limited to, the group consisting of Alzheimer's Disease, Down's Syndrome, anencephaly, spina bifida, meningocele, meningomyelocele, holoprosencephaly, and pancreatic disease.

15 The invention additionally includes the method as above, wherein the condition exists as a prenatal condition.

 The invention additionally includes a method of diagnosing the presence of AD in a human subject suspected of having AD which comprises:

- 20 (a) incubating a biological sample from said subject suspected of containing nPTP with a molecule capable of identifying nPTP; and
- (b) detecting the molecule which is bound in the sample, wherein the detection indicates that the subject has AD.

25 The invention additionally includes a method of diagnosing the presence of DS in a human subject suspected of having DS which comprises:

- 30 (a) incubating a biological sample from said subject suspected of containing nPTP with a molecule capable of identifying nPTP; and
- (b) detecting the molecule which is bound in the sample, wherein the detection indicates that the subject has DS.

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The invention additionally includes a method of diagnosing the presence of pancreatic disease in a human subject suspected of having pancreatic disease which comprises:

5 (a) incubating a biological sample from said subject, which is suspected of containing PTP, in the presence of a binding molecule capable of identifying PTP; and

(b) detecting molecule which is bound in the sample, wherein the detection indicates that the subject has pancreatic disease.

10 The invention additionally includes the methods as above, wherein a biological sample is removed a human subject prior to contacting the sample with the molecule.

The invention additionally includes the methods as above, wherein detecting any of the molecules bound to the protein is performed by in situ imaging.

15 The invention additionally includes the methods as above, wherein detecting of any of the molecule bound to the protein is performed by in vitro imaging.

20 The invention additionally includes the methods as above, wherein the biological sample is reacted with the molecule in a manner and under such conditions sufficient to determine the presence and the distribution of the protein.

25 The invention additionally includes the methods as above, wherein a detectably labeled binding molecule is administered to a human subject.

The invention additionally includes the methods as above, wherein the molecule is bound to the protein in vivo.

30 The invention additionally includes nPTP substantially free of any natural impurities with molecular weights of less than about 17 kD daltons, the nPTP having a molecular weight of about 17-20 kD. However, larger molecular weight forms may be detected and isolated with more vigorous extraction procedures.

The invention also includes a method for recovering nPTP substantially free of natural impurities which includes, but is not limited to, the following steps:

- 5 (a) recovering crude nPTP from a biological sample;
- (b) 10 subjecting the crude nPTP from step (a) to ion-exchange chromatography to obtain partially purified fractions of nPTP;
- (c) 15 subjecting the partially purified fractions of nPTP from step (b) to molecular sieve chromatography to obtain nPTP; and
- (d) 20 purifying nPTP to homogeneity by subjecting the nPTP from step (c) to gel chromatography to obtain nPTP substantially free of natural impurities with molecular weights of less than about 17 kD, said nPTP having a molecular weight of about 17-20 kD daltons.

The invention also includes the method as above, further comprising:

- 20 (e) subjecting the purified nPTP obtained in step (d) to affinity chromatography to obtain highly purified nPTP substantially free of natural impurities with molecular weights of less than about 17 kD, the nPTP having a molecular weight of about 17-20 kD.

25 The invention is particularly directed to a diagnostic method for determining the presence of AD in a human subject by detecting and measuring the concentration of nPTP by immunoassay, comprising:

- 30 (a) reacting a biological sample from a subject suspected of containing nPTP with an antibody or antibodies specific to nPTP;
- (b) monitoring the reaction of step (a) to determine whether the antibodies have bound to nPTP, the concentration of nPTP indicating whether the subject has AD.

The invention is also directed to a diagnostic method for determining the presence of DS in a human subject by detecting and measuring the concentration of nPTP by immunoassay, comprising:

- (a) reacting a biological sample from a subject suspected of containing nPTP with an antibody or antibodies specific to nPTP;
- (b) monitoring the reaction of step (a) to determine whether the antibodies have bound to nPTP, the concentration of nPTP indicating whether the subject has DS.

Additionally, the invention is particularly directed to a diagnostic method for determining the presence of pancreatic disease in a human subject by detecting and measuring the concentration of PTP by immunoassay, comprising:

- (a) reacting a biological sample from a subject suspected of containing PTP with an antibody or antibodies specific to PTP;
- (b) monitoring the reaction of step (a) to determine whether the antibodies have bound to nPTP, the concentration of PTP indicating whether the subject has pancreatic disease.

The present invention also particularly relates to the diagnostic methods recited above, wherein the immunoassay comprises two different antibodies bound to a solid phase support combined with a third different detectably labeled antibody in solution.

DESCRIPTION OF THE FIGURES

Figure 1: Comparison of nPTP concentrations in various regions of normal and AD brain. High levels of nPTP were

found in all areas of AD brain tested. Alternate expression of nPTP immunoreactivity as ng/mg weight (top), ng/mg protein (middle), and ng/mg DNA (bottom) did not alter the magnitude of values observed in AD brain.

5 Figure 2: Epitope mapping of PTP derived from pancreatic fluid compared to PTP in Area 20/21 of AD brain. The mAbs designated 7, 9 and 10 were bound to a solid support and incubated with PTP or AD brain extract. Immunoreactivity was detected with ¹²⁵I-labeled mAbs 7*, 9*, and 10* (see Gross *et al.*, *J. Clin. Invest.* 76:2115-2126 (1985)). All three
10 epitopes present on the native pancreatic form of PTP were found in AD brain extracts.

15 Figure 3: Quantitative soluble nPTP levels measured by a three site M-IRMA in various brain regions of normal controls (NC), AD patients and disease controls (DC). The shaded area represents the range of nPTP values found in NC and DC brains. One AD brain had normal levels of nPTP in regions CB, 11, 17 and 8/9. However, the histopathologic changes of AD in this brain were only observed in the hippocampus.

20 Figure 4: Molecular size of central nervous system nPTP in 4 AD brains compared to purified nPTP standard and PTP in pancreatic fluid (PF). The apparent molecular weight of the taller nPTP peak is 14.4 kD and the shorter peak is approximately 26 kD (hatched areas). AD brain nPTP immunoreactivity
25 had a range between 17 and 20 kD and all 4 subjects have the same species.

30 Figure 5: Distribution and number of neurons bearing neurofibrillary tangles (NFT) and nPTP immunoreactivity in AD, DS, and normal brains. Various brain regions were analyzed and the results expressed as the number of positive staining cells per 20 fields at 250X. HC = hippocampus, CB = cerebellum.

Figure 6: Immunoperoxidase staining of frontal cortex derived from AD and normal brain. The top panel represents

cells from AD brain stained with: mAb 9 for the presence of nPTP (A) and NFTs (B) with a polyclonal antiserum. The bottom panel represents PTP (C) and NFTs (D) immunoreactivity of normal brain. The arrows indicate positive staining cells. The large neurons that stained positive for NFTs were also highly immunoreactive for nPTP (compare A and B). Comparable regions of control brain had no such immunoreactivity.

Figure 7. Quantitative measurement of soluble nPTP levels in CSF derived from 12 subjects with AD compared to two normal control subjects. mPTP levels in CSF of some AD patients are strikingly elevated.

Figure 8. Molecular sizes of nPTP in CSF derived from a patient with AD compared to PTP from pancreatic fluid. There are three peaks of immunoreactivity. One form (major peak) co-migrates with the pancreatic form of the protein. Two other smaller peaks with M.W. varying between 17 and 20 kD are also present (hatched areas).

Figure 9. cDNA and deduced amino acid sequence of bovine PTP. A bovine pancreatic cDNA library ligated into the EcoRI site of the λ ZAP cloning vectors (Lang) was screened with polyclonal antibody to purified PTP. Twenty-seven clones with insert sizes between 0.65 and 0.9 kB were identified from 6×10^4 plaques. A probe prepared from the 2-1 insert hybridized with 20 of the 27 clones by Southern analysis. The 2-1 insert was sequenced by the dideoxynucleotide chain-termination method using plasmid DNA and T7 polymerase (Ausubel, F.M., et al., Current Protocols in Molecular Biology, Wiley & Sons, New York, 1989, Chapter 7.4). This full-length clone has one continuous open reading frame beginning from the first methionine codon and a polyadenylation signal. The deduced